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Allelopathic effects of *Paeonia decomposita* on seed germination and protective enzymes activities of wheat

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The root, stem and leaf aqueous extracts of *Paeonia decomposita* were assayed at 0.025, 0.050, 0.075 and 0.1 g ml^{-1} for their seed germination and early seedling growth of wheat. All aqueous extracts at all concentrations inhibited seed germination and seedling growth of wheat compared with the control, and the degree of inhibition increased with the incremental extracts concentration. Protective enzymes unfolded different trends with increasing concentrations of aqueous extracts. Compared to the control, superoxide dismutase (SOD) and polyphenol oxidase (PPO) activity increased at first but then dropped with increasing concentrations of root extracts, but increased with increasing concentrations of stem extracts. Catalase (CAT) activity increased with increasing concentrations of all the aqueous extracts. It was concluded that *P. decomposita* extracts, especially in root, have significant herbicidal effects on the germination and growth of wheat, and the mechanisms of antioxidative enzymes under allelopathic effects of *P. decomposita* on wheat are not yet clear.

Key words: *Paeonia decomposita* Hand.-Mazz., allelopathic effects, wheat, protective enzyme.

INTRODUCTION

The antagonistic effects of certain tree species such as walnut tree (*Juglan* spp.) on understorey plants and nearby crops were known to humans, centuries ago (Willis, 2000, 2004; Jabeen et al., 2011) and these plants may interfere with the growth of neighboring plant through competition and/or allelopathy.

Allelopathy is an interference mechanism in which living or dead plants release allelochemicals exerting a negative or positive effect on surrounding plants and microorganism, and thus can govern the community dynamics, pattern and productivity in natural and agroecosystem (Rice, 1984; Willis, 2000; Duke et al., 2001; Irshad and Cheema, 2004). A great deal of allelopathic research has been conducted in various fields of agricultural and biological sciences and a number of plant species have been reported to have an allelopathic effect on other plant species (Kato-Noguchi, 2003; Jefferson and Pennacchio, 2003; Oueslati, 2003; Djurdjevic et al., 2004).

Allelochemicals produced by one species can influence the growth, productivity, and yield of other or the same species (Batish et al., 2001). The avoidance of allelopathic effects between species, or the exploitation of beneficial interactions in a rotation or a mixed cropping system may have direct bearing on the crop yield (Rizvi et al., 1992).

Paeonia decomposita Hand.-Mazz., a wild species of tree peonies is a member of the family Paeoniaceae that is native to Southwest China is a perennial deciduous shrub with great medicinal and ornamental values (Wang and Xie, 2004). It also is of genetic importance to the plant breeding and study of peony evaluation. Therefore, *P. decomposita* exhibits economic potential for both herbal and floriculture exploitation. However, a relatively reduced understorey is often observed below it. We suspected an allelopathic mechanism in *P. decomposita* that might affect the susceptible species. In the present study, the allelopathic effects of roots, stem and leaf

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aqueous extracts of *P. decomposita* were examined to determine if inhibitory or stimulatory effects of *P. decomposita* extracts influence seed germination and seedling growth of wheat that is commonly used in bioassay of allelopathic effects.

MATERIALS AND METHODS

Location

P. decomposita plants were collected from fields (30° 56´N, 101° 44´E) in Danba country of Sichuan province in June, 2011, where there is a natural range of *P. decomposita*. The experiment was carried out at the Department of Landscape Architecture, Sichuan Agricultural University, China, from June to October, 2011.

Preparation of extracts

Fresh *P. decomposita* plants were separated into leaves, stems and roots. The stems and leaves were chopped into 1 cm long pieces and roots were chopped into 0.5 cm thick slices. The components were oven dried at 50°C for 5 days and then milled and passed through a 40 mesh sieve. Fifty grams of dried roots, stems and leaves were, respectively extracted by soaking in 500 ml of distilled water with mild stirring at 25°C for 24 h. The collected leachate was filtered through cheese cloth to remove debris and finally filtered using Whatman No.1 filter paper to have 0.1 g ml^{-1} concentration. Fresh stock extracts were kept in a refrigerator at 4°C until used.

Seed bioassay

Stock extracts (root, stem and leaf) were diluted with sterile distilled water to give final concentrations of 0, 0.025, 0.050, 0.075 and 0.1g ml^{-1}. Wheat seeds (*Triticum durum*) were sterilized with 2% sodium hypochlorite for 15 min and then washed with doubled distilled water 3 times. Thirty uniform seeds of wheat were germinated in sterilized petri-dishes lined with two layers of Whatman No. 1 filter paper and moistened with 5 ml of respective root, stem or leaf leachate concentration in treatment and distilled water in control. Each treatment had five replicates and each experiment was repeated 3 times. The petri-dishes were incubated at 25 ± 2°C in growth chamber. After three days of culture, germination percentages were recorded and the radicle and coleoptile length were measured. A seed was regarded as germinated when its radicle was ~2 mm in length. At the same time, the plants were harvested, washed with water, and the leaves were stored in Zip-Loc bags in a freezer at -20°C for subsequent analysis.

Measurements of protective enzymes activities

For determination of protective enzymes activities, 0.2 g frozen plant material was homogenized in 1 ml of ice-cold 0.05 mol L^{-1} phosphate buffer (pH 7.8) containing 1% polyvinylpyrrolidone (PVP). The homogenate was then centrifuged for 15 min at 12,000 g. A portion of the eluent was analyzed immediately for catalase activity, and the remainder was stored at -20°C for subsequent analysis of superoxide dismutase (SOD) and peroxidase (POD).

SOD activity was measured by monitoring the inhibition of nitroblue tetrazolioum (NBT) reduction at 560 nm according to Li (2000). The reaction mixture contained 0.05 mol L^{-1} phosphate buffer (pH 7.8), 0.1 mmol L^{-1} tetrasodium-ethylenediaminetetraacetate (Na-EDTA), 75 µmol L^{-1} riboflavin, 13 mmol L^{-1} methionine and 0.01 to 0.05 ml enzyme extract. Reaction was carried out in test tubes at 25°C under a fluorescent lamp (40 W) with irradiance of 75 µmol m^{-2} s^{-1}. The reaction was allowed to run for 15 min and stopped by switching the light off. Blanks and controls were run in the same manner but without irradiation and enzyme, respectively. Under the experimental condition, the initial rate of reaction, as measured by the difference in the increase of absorbance at 560 nm in the presence and absence of extract was proportional to the amount of enzyme. The unit of SOD activity was defined as the amount of enzyme that inhibited the NBT photoreduction by 50%. SOD activity was valued in units per gram of plant material.

Catalase activity was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 2 ml of 3% H_2O_2, 1.9 ml of distilled water and 0.1 ml extract (Hao et al., 2004). Reaction was started by adding 0.1 ml enzyme extract. The CAT activity was expressed in U g^{-1} fresh weight (FW) and one unit was defined as equal to a change of 1 in absorbance per min.

Polyphenol oxidase (PPO) activity was measured by the method of Kumar and Khan (1982). The assay mixture for PPO contained 2 ml of 0.1 M phosphate buffer (pH 6.0), 1 ml of 0.1 M catechol and 0.5 ml of enzyme extract. This was incubated for 5 min at 25°C, after which the reaction was stopped by adding 1 ml of 2.5 N H_2SO_4. The absorbance of the purpurogallin formed was read at 495 nm. 2.5 N H_2SO_4 was added to the blank at the zero time of the same assay mixture. The activity of PPO was also expressed in units g^{-1} FW and one unit was defined as equal to a change of 0.01 in absorbance per min.

Data analysis

Germination percentage, the radicle and coleoptile length data and protective enzyme activity data are expressed as means ± standard deviation (SD) of three replicates. One-way analysis of variance (ANOVA) was used to compare the effects of different concentration of aqueous extracts and the effects of different aqueous extracts at same concentration. Differences between means were considered to be significant at $P \leq 0.05$ by the least significant difference (LSD) test. Statistical analyses were done with SPSS13.0 for Windows.

RESULTS

Effect of aqueous extracts of *P. decomposita* plants on germination of wheat

Wheat seeds germinated completely after 36 h incubation in distilled water and the germination was 100%. Over control, the germination percentage of wheat seeds decreased with increasing concentration of aqueous extracts of *P. decomposita* (Figure 1). Aqueous extracts of root had the most restrictive to wheat seed germination and germination decreased by 20, 36.67, 56.22 and 66.07% at 0.025, 0.050, 0.075 and 0.1 g ml^{-1}, respectively, while the decrease was by 3.33, 16.67, 36.67 and 43.33% at 0.025, 0.050, 0.075 and 0.1 g ml^{-1}, respectively for extracts of stems.

Effect of aqueous extracts of *P. decomposita* on seedling growth

Increased concentration of aqueous extracts from all plant parts inhibited seedling growth of wheat. The degree

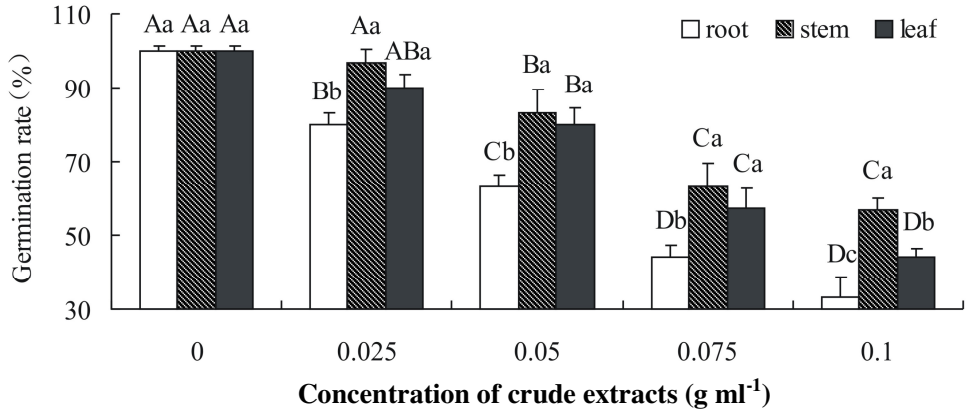

Figure 1. Effects of extracts of *Paeonia decomposita* plants on germination of wheat.
Mean ± standard deviation, the different capital letters (for example, A, B, C, D) on the bar indicated significant difference among different concentrations of the same plant tissue at the 0.05 level by LSD, while the different lowercase letters (a, b, c) indicated significant difference among different plant tissues of the same concentration at the 0.05 level by LSD.

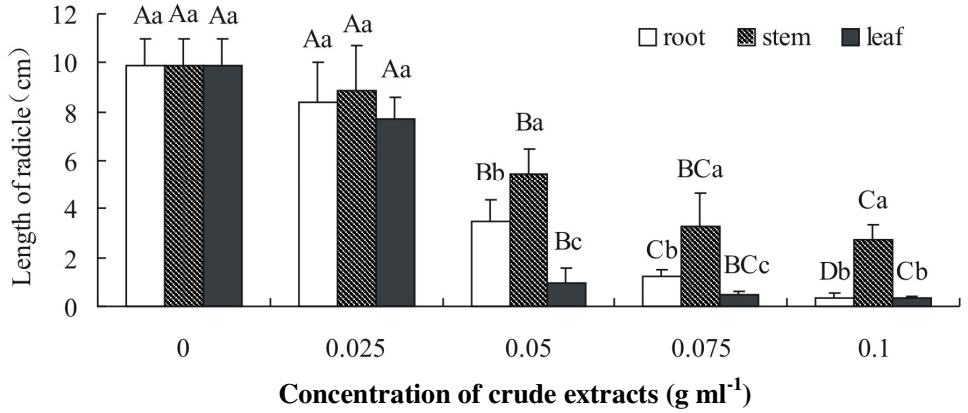

Figure 2. Effects of extracts of *Paeonia decomposita* plants on length of radicle of wheat seedling.
Mean ± standard deviation, the different capital letters (for example, A, B, C, D) on the bar indicated significant difference among different concentrations of the same plant tissue at the 0.05 level by LSD, while the different lowercase letters (a, b, c) indicated significant difference among different plant tissues of the same concentration at the 0.05 level by LSD.

degree of inhibition increased with increasing extract concentration. Significant inhibitory effects on radicle length were found in medium containing 0.050 g ml^{-1} extract or above as compared to the control. At 0.050 g ml^{-1} leachate of root, stem and leaf, the radicle lengths were reduced to nearly 35.07, 54.90 and 9.37%, respectively (Figure 2).

Significant differences were found among different plant tissues at 0.050 and 0.075 g ml^{-1}. At the highest extract concentration (0.1 g ml^{-1}), the radicle length in medium containing stem extract was significantly higher than that of root and leaf (Figure 2). All aqueous extracts at all concentrations inhibited coleoptile length compared with the control, and the degree of inhibition increased with increasing extract concentration, especially for leachate of root and leaf (Figure 3). At the highest extract concentration (0.1 g ml^{-1}), the coleoptile length were reduced to nearly 3.40, 27.40 and 3.53%, respectively.

Effect of aqueous extracts of *P. decomposita* on protective enzymes activities

Compared to the control, SOD (Figure 4) activity in wheat seedlings evaluated in this study increased at first but then dropped with increasing concentrations of aqueous extracts of root and leaf, and both achieved highest figures at 0.075 g ml^{-1}, while SOD activity in wheat seedlings treated with stem extracts increased with increasing concentrations. At the same concentration,

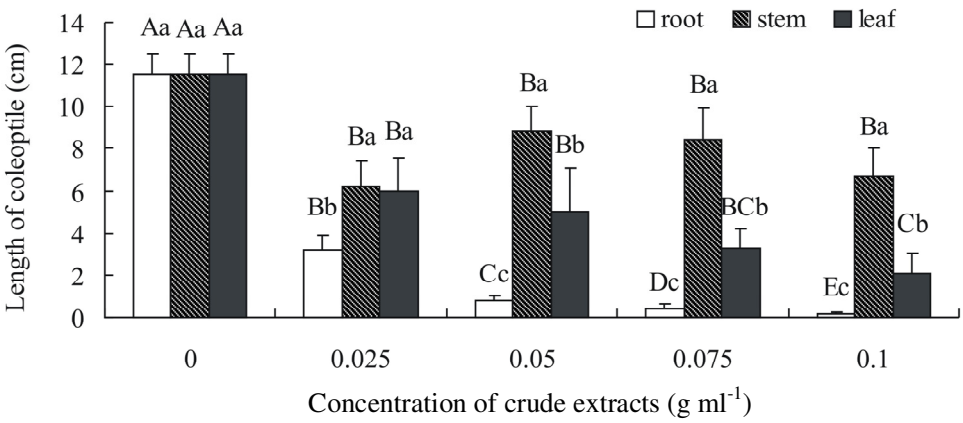

Figure 3. Effects of extracts of *Paeonia decomposita* plants on length of coleoptile of wheat seedling.
Mean ± standard deviation, the different capital letters (for example, A, B, C, D) on the bar indicated significant difference among different concentrations of the same plant tissue at the 0.05 level by LSD, while the different lowercase letters (a, b, c) indicated significant difference among different plant tissues of the same concentration at the 0.05 level by LSD.

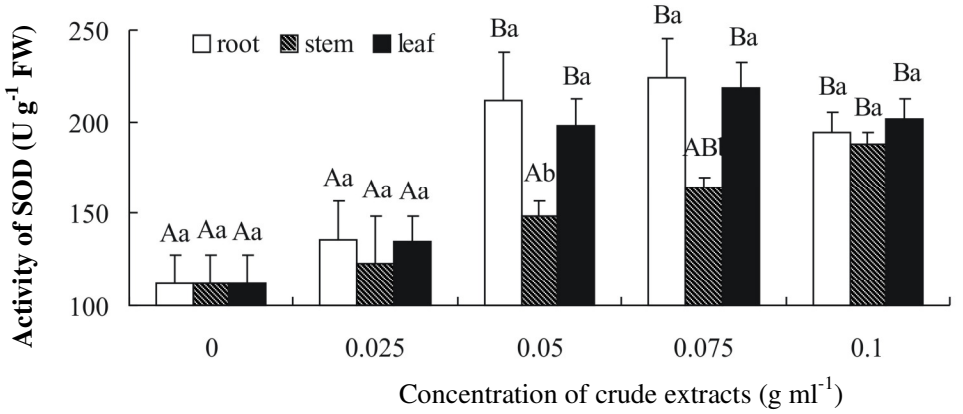

Figure 4. Effects of extracts of *Paeonia decomposita* plants on SOD activity of wheat seedling.

SOD activity in wheat seedlings treated with stem extract were usually lower than that of seedlings treated with root extract and leaf extract, and the difference was significant at 0.050 and 0.075 g ml^{-1}. CAT (Figure 5) activity unfolded a similar tendency, all of them increased with increasing concentrations of aqueous extracts and at the same concentration, CAT activity in wheat seedlings treated with root extract were usually higher than that of seedlings treated with stem extract and leaf extract, and the difference was significant except at the control level.

PPO (Figure 6) activity in wheat seedlings evaluated in this study unfolded a similar tendency with SOD activity, it increased at first but then dropped with increasing concentrations of root extracts and had the highest activity at 0.075 g ml^{-1}. But PPO activity in wheat seedlings treated with stem extract and leaf extract increased with increasing concentrations and had the highest activity at 0.1 g ml^{-1}. Seedlings treated with stem extract had the lowest PPO activity and seedlings treated with root extract had the highest at 0.025, 0.050 and 0.075 g ml^{-1}. At 0.1 g ml^{-1}, there was no significant difference between PPO activities of seedlings treated with root extract and leaf extract, but both of them were higher than that of seedlings treated with stem extract.

DISCUSSION

There are evidences that plants may interfere with the growth of neighboring plant through exuding inhibitory substances (Bais et al., 2003). In the rhizosphere soil and root bark of *Paeonia ostii*, five phenolic compounds of ferulic acid, cinnamic acid, vanillin, coumarin and paeonol were detected and some of them were reported previously

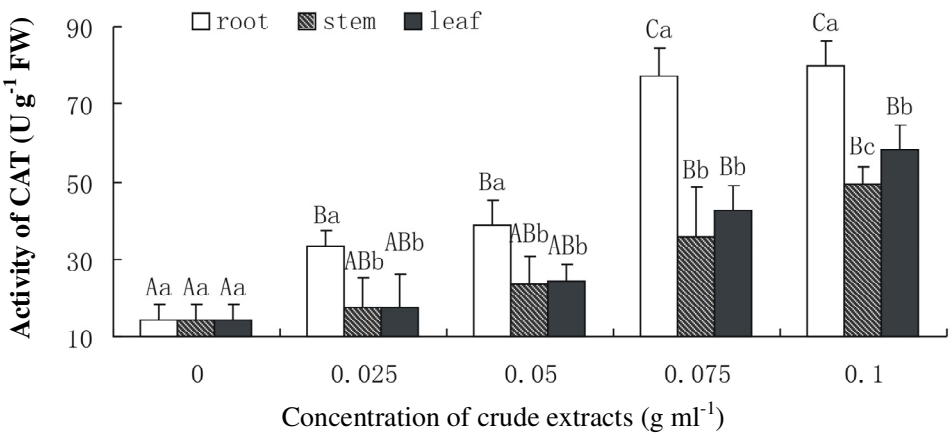

Figure 5. Effects of extracts of *Paeonia decomposita* plants on CAT activity of wheat seedling.

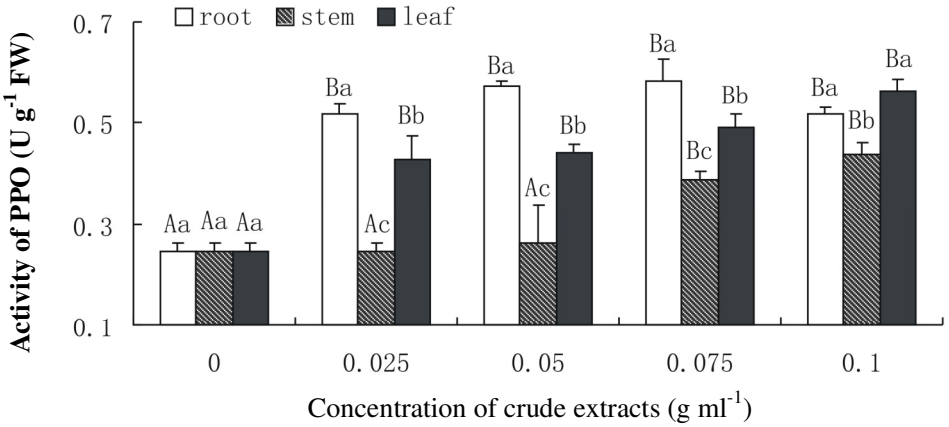

Figure 6. Effects of extracts of *Paeonia decomposita* plants on CAT activity of wheat seedling.

as allelopathic substances (Qin et al., 2009).

In this work, germination of wheat seeds and seedling growth were considerably affected by aqueous extracts of root, stem or leaf tissues of *P. decomposita*. This was consistent with that of *P. ostii* (Qin et al., 2009) which was found to contain allelopathic substances. At the same concentration, root extract usually had the most restrictive effect, and inhibitory effect of stem extract was the weakest. This observation however was contrary to that of Otusanya et al. (2007) who found that the degree of retardatory effects of the shoot aqueous extracts of *Amaranthus cruentus* was greater than that of root aqueous extracts. In ginger plants, Han et al. (2008) observed that the degree of toxicity of different plant parts can be classified in order of decreasing inhibition as stem > leaf > rhizome. This indicated that the amount of potency of allelochemicals were considerably different in different plants.

The radicle and coleoptile growth of germinating wheat seedlings treated with aqueous extract from different *P. decomposita* plant parts were observed to be inhibited. This was consistent with that of Kamal (2011) who found allelochemicals secreted by sunflower inhibited shoot length, and root length of wheat seedlings. Nie et al. (2005) explained that aqueous extracts of different organs significantly inhibited the activity of nitrate reductase and glutamine synthetase, and inhibited the growth of the root and its activity. This not only restricted the absorption of water and nutrition by root system, but also had negative effect on the healthy growth of the aerial part.

Increasing in protective enzyme activities ensured that the plants treated can effectively protect the membrane from the active oxygen and led to the stability of the membrane, so that the seedlings were able to acclimated to the allelopathic stress by adjusting the activities of SOD, CAT and PPO. As to root extract, SOD activity and PPO activity increased at first but then dropped with increasing concentrations of aqueous extracts, and SOD activity showed similar tendency to leaf extract. But to

stem extract, SOD, CAT and PPO activity increased with increasing concentrations of aqueous extracts and CAT activity to root extract; PPO activity to leaf extract also showed similar tendency. It was indicated that roots of *P. decomposita* had more relatively allelopathic substances than that of stem and leaf. The mechanisms of antioxidative enzymes under allelopathic stresses are not yet clear.

Conclusion

P. decomposita extracts have significant herbicidal effects on the germination and growth of wheat. Crop residues of this species could be spread on wastelands, resulting in the leaching of allelopathic substances that would reduce possible weed density in agricultural fields and forest. However, further studies are needed to isolate and identify the active compounds from *P. decomposita* that might be exploited for plant disposition management.

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